Effects of two kinds of transgenic poplar on protective enzymes system in the midgut of larvae of American white moth

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Abstract: The leaves of Bt (*Bacillus thuringiensis*) transgenic poplar (*Populus nigra* L.) and CpTI (Cowpea trypsin inhibitor) transgenic poplar ((*P. tomentosa*×*P. bolleana*)×*P. Tomentosa*) were taken to feed the 4th-5th-instar larvae of American white moth (*Hyphantria cunea* (Drury)) for determination of the activities of the protective enzyme system inside larvae's body. The physiological and biochemical effects of the transgenic poplars on the larvae were studied. The results showed that the two kinds of transgenic poplars had similar effects on the protective enzyme system in the midgut of larvae. The activities of superoxide dismutase, catalase, and peroxidase in midgut of the larvae increased gradually, reached the highest value at a certain time, and then decreased suddenly. For the larvae that were fed with the leaves of Bt transgenic poplar, the peak value of superoxide dismutase and catalase presented at the time of 24-h feeding, while the peak of peroxidase took place at the time of 12-h feeding. The activities of these protective enzymes for the larvae that were fed with leaves of CpTI transgenic poplar peaked 12 h later than that of those fed with leaves of Bt transgenic poplar. The comparison of activities of the protective enzymes was also carried out between the larvae with different levels of intoxication. It was found that the activities of protective enzyme of the seriously intoxicant larvae were higher than that of the lightly intoxicant larvae. This difference was more obvious in the group treated with CpTI transgenic poplar.

Keywords: Transgenic poplar; American white moth; Protective enzyme system
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Introduction

It is common knowledge that reactive oxygen is a kind of metabolite consisting in oxygen demand organism. Under normal physiological condition, the reactive oxygen content is in low level. Decrepitude, illness, and stress can affect the respiratory chain of mitochondria and the electron-transmit chain of microsome so that superoxide radical engender excessively (Fang *et al.* 1989; Zhao 1993; Zhang 1992). Enzymes of protective system include Superoxide dimutase (SOD), Catalse (CAT), and Peroxidase (POD) (Fridovich 1977), and they defense oxidative damage in organism.

SOD can turn O_2^- to H_2O_2 through dismutation, then, CAT and POD turn H_2O_2 into H_2O. This a series of reaction can eliminate biomembrane damaged by the O_2^-. With regard to the enzyme activities of protective system, many researches have been done on plant, but few reports were found on insect, especially the effects of transgenic plant on insects.

Materials and Methods

Plant materials

The leaves of Bt transgenic poplar (*Populus nigra L.*) were provided by Hanyifan Research Group, Chinese Academy of Forestry. CpTI transgenic poplar ((*P. tomentosa*×*P. bolleana*) ×*P. tomentosa*) was owned by our laboratory.

Insect materials

Larvae of American white moth (*Hyphantria cunea* Drury) were collected form the trees along street in Kuandian county, Liaoning Province and reared more than one day. 4th~5th-instar larvae, growing normally, were selected. They were fed with leaves of the transgenic poplar after

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being hungry for 4 h. The activities of protective enzyme were studied through dissection of midguts of larvae

Assay methods

Enzyme extract:

Put midguts of larvae into pre-chilled PBS buffer (0.05 mol/L, pH7.0, 1% PVP). Grinded in ice bath, then centrifuged in 20,000 rpm under 2℃ for 20 min. The supernatant fluid was enzyme extract.

Enzyme activities determination:

Activity of SOD determination (Fang *et al.* 1989): Added 50-μL diluted enzyme extract into 3.0-mL reaction medium (PBS 0.05 mol/L pH 7.8 containing 77.12 μmol/L NBT, 0.1 mmol/L EDTA and 13.37 mmol/L Met), then, added 0.4 mL 0.2 mmol/L riboflavine. Reacted under 4,000lux illumination condition for 15 min. After that, stopped the reaction by dark. Colorimetric analysis was in 560 nm. Enzyme activity of SOD=$(A_0-A)\times V_0/(A_0\times W\times T\times V\times 50\%)$. A and A_0 stand for the OD value of sample and blank respectively. V_0 and V stood for the total volume of the enzyme and the volume in the reaction system. W was total mg protein. T was reaction time.

Activity of CAT determination (Chance & Machly 1995): Added 100-μL diluted enzyme extract into 5.0-mL reaction medium (PBS 0.05 mol/L pH7.0 containing 8 mmol/LH_2O_2). Reacted under 30℃ for 1 min, then stopped the reaction by 2.0-mL 10%H_2SO_4. Titrated surplus H_2O_2 with 2-mmol/L $KMnO_4$.

Activity of POD determination (Simon *et al.* 1974): Added 150-μL diluted enzyme extract into 3.0-mL reaction medium (PBS 0.1mol/L pH6.0 containing 30-mmol guaiacol, 26-mmol H_2O_2). Reacted for 10 min. Colorimetric analysis was in 470 nm. The value of OD_{470} increased 0.01 was defined one unit of POD activity.

Protein content of enzyme extract was determined by the method of Coomassie brilliant bull G250.

Results and analyses

Effects of Bt transgenic poplar on the enzyme activities of protective system

Selected the normally growing 4th~5th-instar larvae and fed them with leaves of Bt transgenic poplar. The activities of SOD, CAT and POD in the midgut of larvae were determined after 4, 12, 24, 36 and 48 h (see Table 1).

Table 1. Effects of Bt transgenic poplar on SOD, CAT and POD of the American white moth larvae

Time /h	Activity of SOD unit·mg^{-1}·min^{-1}	Activity of CAT umol·mg^{-1}·min^{-1}	Activity of POD unit·mg^{-1}·min^{-1}
0	2.53 ± 0.16	1.79 ± 0.23	0.82 ± 0.02
4	3.96 ± 0.23	2.00 ± 0.17	1.07 ± 0.01
12	31.89 ± 2.44	14.78 ± 2.49	7.10 ±0.87
24	80.09 ± 6.98	35.47 ± 5.63	6.74 ± 0.59
36	6.24 ± 0.35	2.19 ± 0.24	0.58 ± 0.15
48	2.90 ± 0.19	2.37 ± 0.26	0.66 ± 0.03

Note: Values are mean ± SD of 3 determinations

Shown as Table 1, as time went on, the activities of SOD and CAT increased gradually and reached the highest value at time of 24-h feeding, then, it went down immediately. The activity of POD had a little difference from SOD and CAT. It peaked 12 hours earlier than SOD and CAT and kept the high value till 24 h, then decreased.

Effects of CpTI transgenic poplar on the enzyme activities of protective system

The selected 4th~5th-instar larvae were fed with CpTI transgenic poplar leaves. The activities of SOD, CAT and POD in the midgut of larvae were measured after 4, 12, 24, 36 and 48 h (see Table 2).

Table 2. Effects of CpTI transgenic poplar on SOD, CAT and POD of the American white moth larvae

Time /h	Activity of SOD unit·mg^{-1}·min^{-1}	Activity of CAT umol·mg^{-1}·min^{-1}	Activity of POD unit·mg^{-1}·min^{-1}
0	2.53 ± 0.16	1.79 ± 0.23	0.82 ± 0.02
4	12.28 ±1.37	5.00 ± 0.96	0.71 ± 0.01
12	24.01 ± 1.94	8.09 ± 1.24	9.60 ± 1.23
24	42.13 ± 3.48	21.48 ± 2.51	20.83 ± 2.56
36	54.40 ± 3.27	24.75 ± 2.24	5.00 ± 0.96
48	4.05 ± 0.65	2.34 ± 0.57	0.61 ± 0.19

Note: Values are mean ± SD of 3 determinations

Shown as Table 2, as time went on, the activity of SOD increased generally and reached the highest value after 36 h. At this time, the activity of SOD was 24.1 fold as much as control. After 36 h, the activity of SOD decreased sharply. The change of CAT activity was just as the same as the SOD. But the activity of POD had a little difference from that of SOD and CAT. Its peak value emerged at the time of 24-h feeding, 12 hours earlier than those of SOD and CAT.

The results as above shown that the enzyme activities of protective system increased with the content of the two kinds of the transgenic poplar leaves that were eaten by the larvae. After reaching the highest value at some time they decreased immediately.

Comparison of enzyme activities between different levels of intoxication in larvae

After 48-h feeding with the leaves of transgenic poplar, most of the larvae moved slowly, but some of them could still move in rapid speed. We selected both slowly moving larvae and fast moving ones to determine their enzyme activities of protective system in their midgut. Table 3 showed the results of comparison of enzyme activities of protective system between the larvae fed with Bt transgenic poplar leaves for 48 h, with different levels of intoxication. The activities of SOD and CAT in the quick-moving larvae were higher than that in the slow ones. But the activity of POD was different from SOD and CAT. The activities of POD in slowly moving larvae were a little higher than that in the quick ones. The reason was not clear. Table 4 showed the comparative results of enzyme activities of protective system between the larvae fed with CpTI transgenic poplar leaves for 48 h and in different levels of intoxication. The activities of SOD, CAT and POD in the quick ones were higher than that in the slow ones.

Table 3. Comparison of activities of SOD, CAT and POD between the larvae fed with leaves of Bt transgenic poplar and in different levels of intoxication

State	Activity of SOD unit·mg^{-1}·min^{-1}	Activity of CAT umol·mg^{-1}·min^{-1}	Activity of POD unit·mg^{-1}·min^{-1}
quick	7.33 ± 0.86	3.69 ± 0.54	0.71 ± 0.26
slow	4.75 ± 0.47	2.82 ± 0.84	1.00 ± 0.19

Note: Values are mean ± SD of 3 determinations.

Table 4. Comparison of activities of SOD, CAT, and POD between the larvae fed with leaves of CpTI transgenic poplar and in different levels of intoxication

State	Activity of SOD unit·mg^{-1}·min^{-1}	Activity of CAT umol·mg^{-1}·min^{-1}	Activity of POD unit·mg^{-1}·min^{-1}
Quick	13.83 ± 1.48	6.89 ± 1.14	5.28 ± 1.06
slow	4.00 ± 0.79	2.04 ± 0.89	0.47 ± 0.07

Note: Values are mean ± SD of 3 determinations

The results as above showed that some larvae still kept the enzyme activities in high level after fed with leaves of transgenic poplar. High-level enzyme activities reduced the damage of the toxic materials, and the larvae were not seriously intoxicated, so they could move quickly. On the other hand, the enzyme activities of protective system in the other larvae were not high enough to eliminate the damage of the toxic material, so that those larvae were intoxicated seriously, moved slowly, even fainted away.

Discussion

The enzyme activities of protective system changed when they were stimulated by exogenous toxic material. When the cabbage worm (*Pieris rapae* Linnaeus), green cochlid (*Latoia consocia* Walker) and brown cochlid (*Setora postomata* Hampson) were treated with decamethrin, the activities of SOD, CAT and POD in the intoxicant insects were higher than that of control, increasing generally with the level of intoxication, and decreasing suddenly when the insects closed to death (Li *et al.* 1994). Treated greater wax moth (*Galleria mellonella* Linnaeus) with *Bacillus thuringiensis* subsp. galleriae, the activities of SOD and POD increased but the activity of CAT did not change (Shen 1993).

In this experiment, the American white moth larvae were fed with two kinds of transgenic poplar, but the change tendency in the activities of protective system in the larvae midgut was same. The activities of SOD, CAT and POD increase generally with time, when reaching the highest value at some time, it decreases sharply. Protective enzyme such as SOD could be induced by the content of subtract (Fang *et al.* 1989). The more leaves of transgenic poplar were eaten by the American white moth, the much serious function that the toxic material in the transgenic poplar would make. Excessive superoxide radical might be made then the protective enzymes such as SOD might be induced. When the content of reactive oxygen amounts to

some threshold value, the synthetic system or activation system of the protective enzymes were damaged, so the enzyme activities went down rapidly after peak value.

The enzyme activities of American white moth with different intoxicant levels were also compared in this experiment. The results showed that under the same feeding condition, the activities of protective enzyme were high in the larvae that were intoxication in low level and moved quickly. On the contrary, the activities of protective enzyme were low in the larvae that were intoxication in high level and moved slowly. It might be one of the important insecticidal mechanism of the transgenic poplar that influence the enzyme activities of protective system.

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